

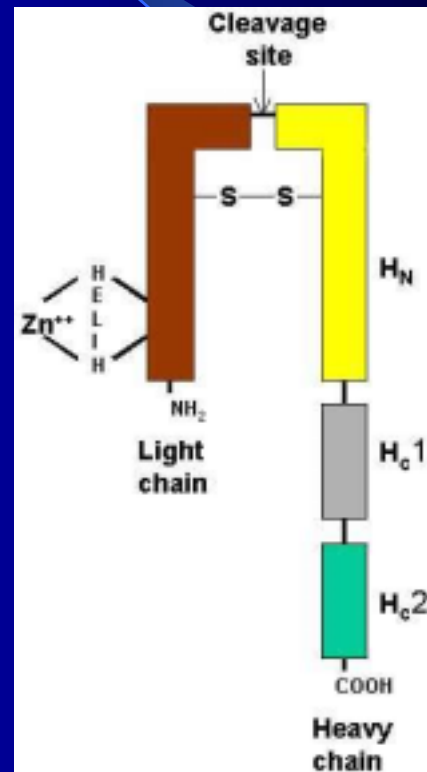
Pharmacological Countermeasures for Botulinum Toxin Exposure

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USAMRICD

Botulinum Neurotoxin (BoNT)

- Most potent substance
- Clostridium botulinum
- Seven serotypes, A-G
- Dichain Protein, 100 kDa
Hc-S-S- 50 kDa Lc
- Foodborne, wound, infant
- Flaccid paralysis
- Cholinergic nervous system



Symptoms of Intoxication

- Symmetric, descending , flaccid paralysis beginning with the cranial nerves
- Diplopia, dysphagia, dysarthria, dysphonia
- Upper extremities, respiratory muscles and lower extremities
- Autonomic:dry mouth blurred vision, constipation
- Onset 18-38 hr (6 hr - 8 days); recovery, months

Roles of BoNT

- Public health threat
- Potential weapon of mass destruction (WMS)
- Tool for selective inhibition of acetylcholine release
- Therapeutic agent for dystonia and movement disorders

Military and Bioterrorism Threat

- Applied as respirable aerosol or food contaminant
- Absorbed from all mucus membranes but not from intact skin
- Can be handled safely by immunized personnel (low risk of self intoxication)
- 8 kg of toxin can produce a lethal cloud over a 100 sq km area.

Professor Emile van Ermangem (1851-1931) University of Ghent



- Investigated outbreak in 1895
- 34 musicians affected, 4 month-old ham implicated
- Described symptoms
- Isolated bacteria, spores
- injection of soluble filtrate reproduced botulism in animals

Foodborne Botulism

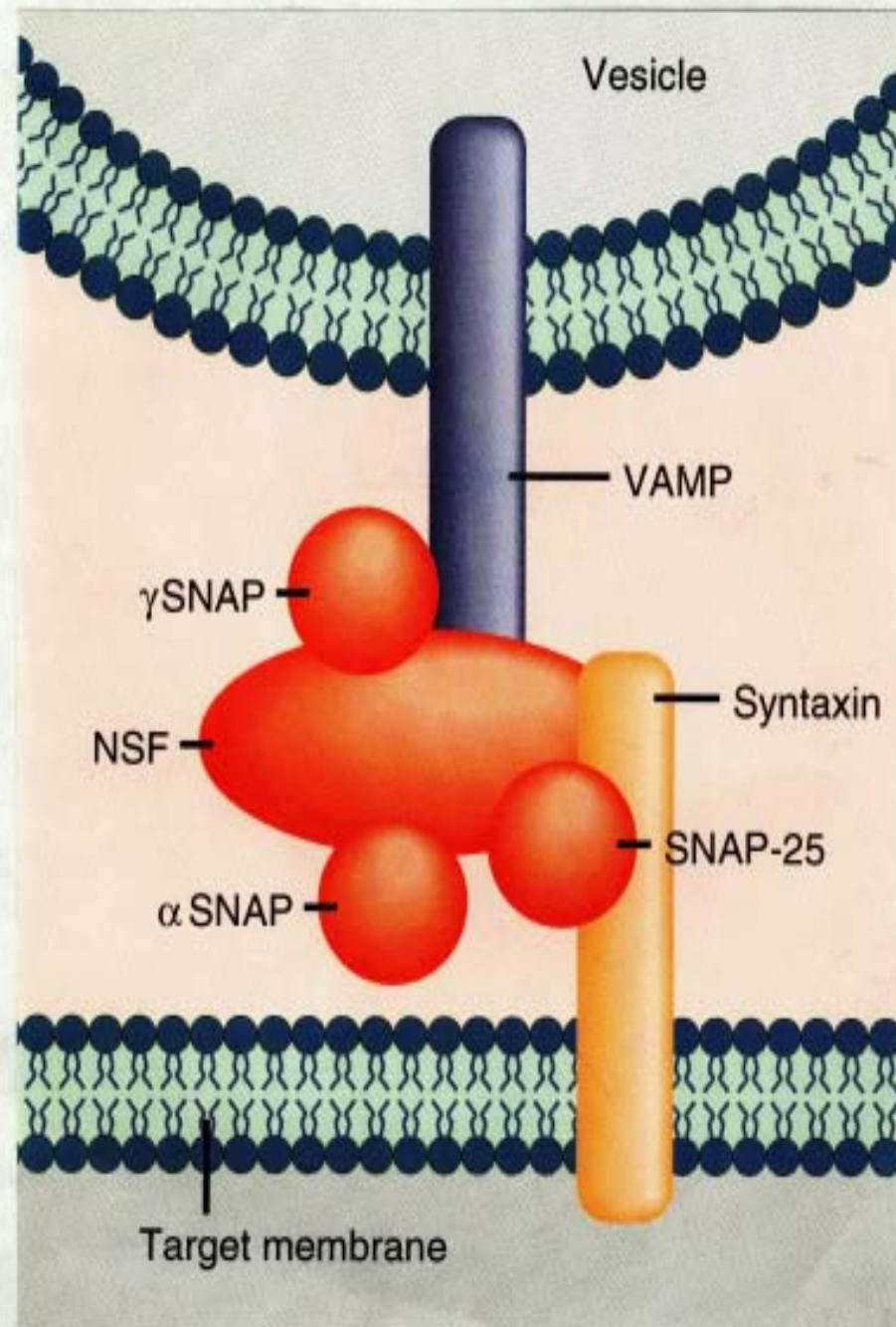
- Intoxication from ingestion of preformed toxin
- Initially associated with meat, especially sausage
- Muller (1870) coined the term botulism (from the Latin word for sausage *botulus*)
- Most human cases are caused by types A, B or E
- Fatality declined from >50% to <10%

Wound Botulism

- First reported in 1943
- Traumatic and surgical wounds
- Growth of *C. botulinum* in necrotic tissues
- Rare: only 47 cases from 1943-1990
- Currently associated with perenteral and intranasal drug abuse

Infant Botulism

- First described in 1976
- Currently the most prevalent form of botulism
- Toxin produced from germination of spores in intestinal tract
- Generally associated with serotypes A and B
- Most common between 3 months and 1 year
- Lack of competing intestinal flora and poorly developed immune system



Strategies for Protection

- Vaccine
- Antitoxin
- Pharmacological treatment

Potential Targets

- Ectoacceptor binding
- Translocation
- Metalloprotease activity

Objectives

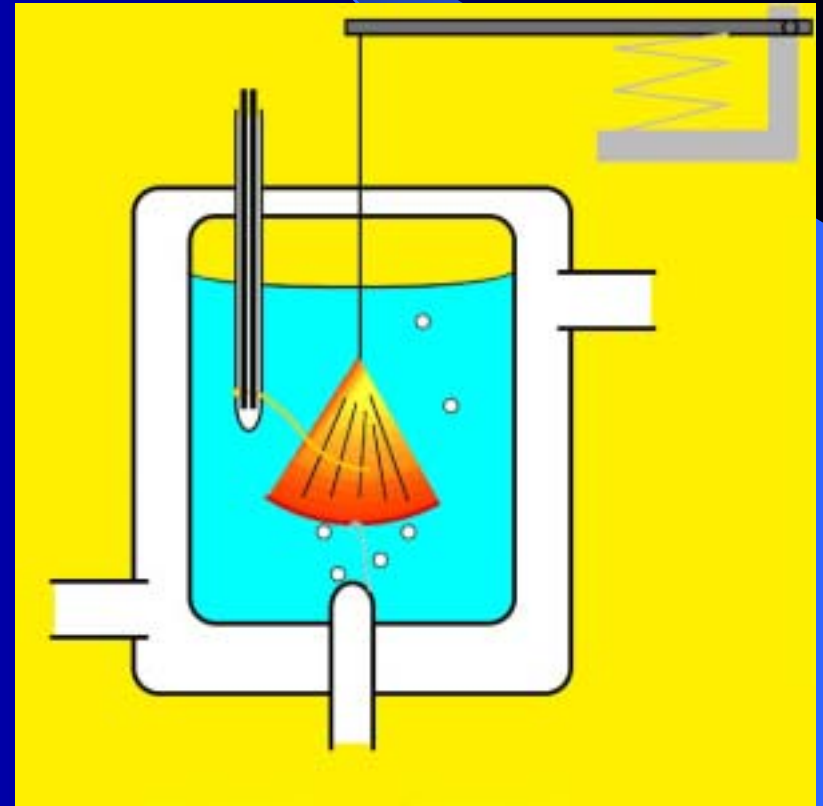
- Develop Drugs
 - Antagonize BoNT-mediated inhibition of binding and internalization
 - Inhibit enzymatic activity of BoNT light chain
- Deliver Drugs
 - Target drug to nerve terminal cytosol
 - BoNT/A heavy chain for targeting and internalization
 - Inert high capacity matrix
 - Reversible binding and dissociation of inhibitors

Test systems

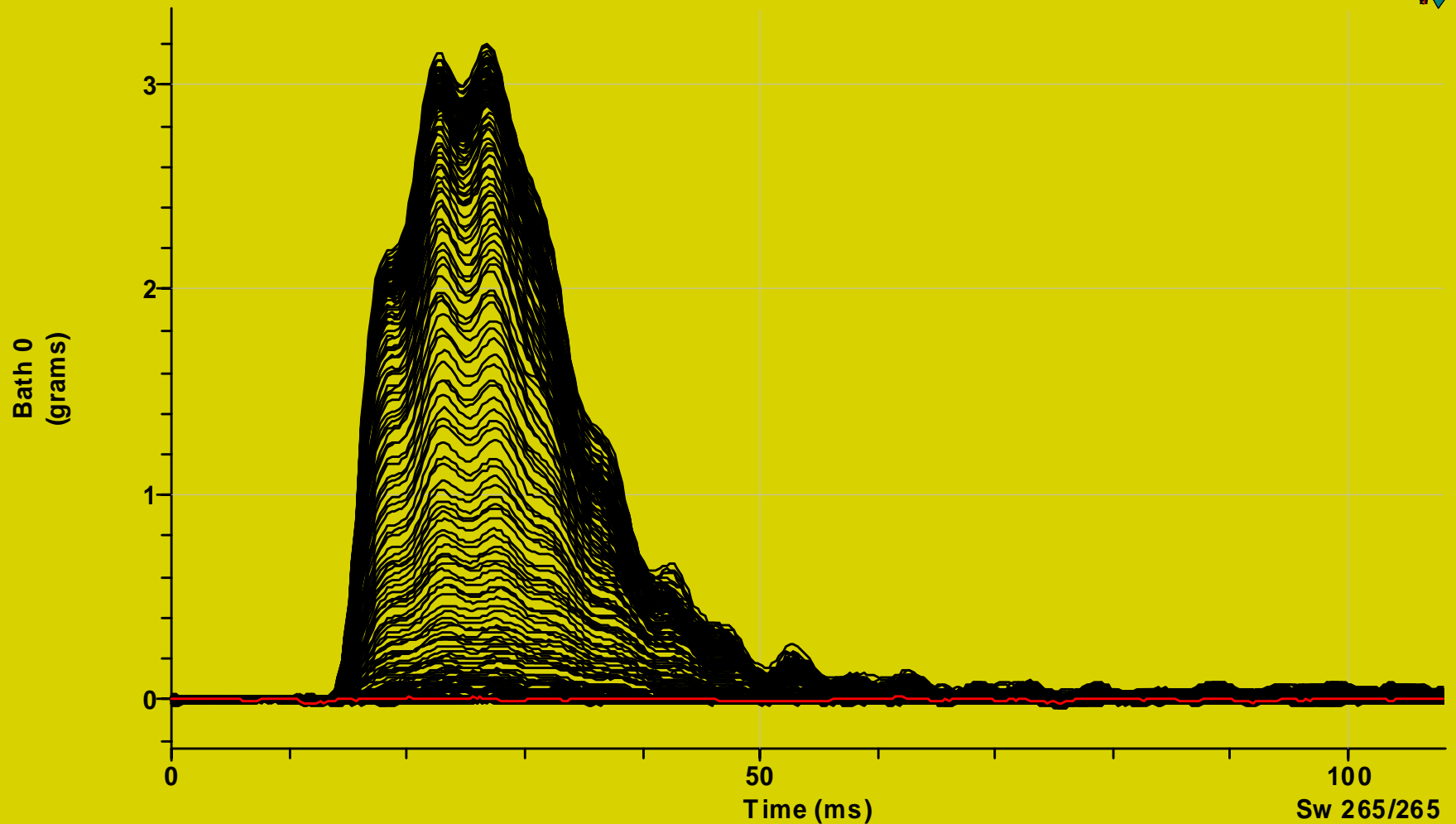
- Cell-free enzymatic assay
 - Capillary electrophoresis
 - Fluorescent microplate
- Cell cultures
 - Primary (spinal cord, CNS)
 - Cell lines (NG108-15, S26)
- Isolated tissues
 - Aplysia californica
 - Mouse diaphragm
- In situ preparations (rat EDL muscle)
- Whole animal toxicity testing (mouse)

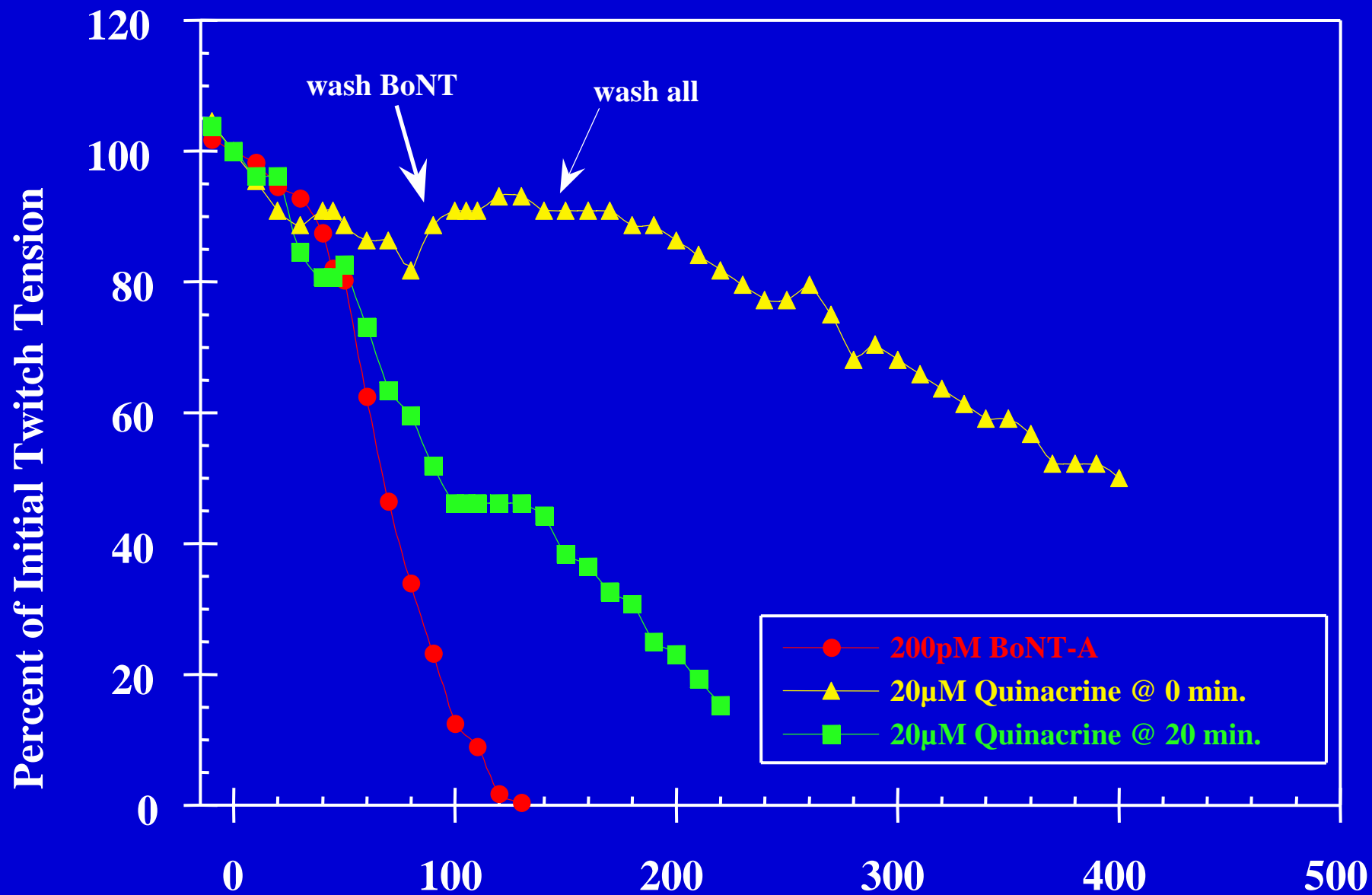
Muscle Tension

- Isolated mouse hemidiaphragm: isometric twitch tension in response to phrenic nerve stimulation.



Depression of Single Twitch (50 pM BoNT/A)

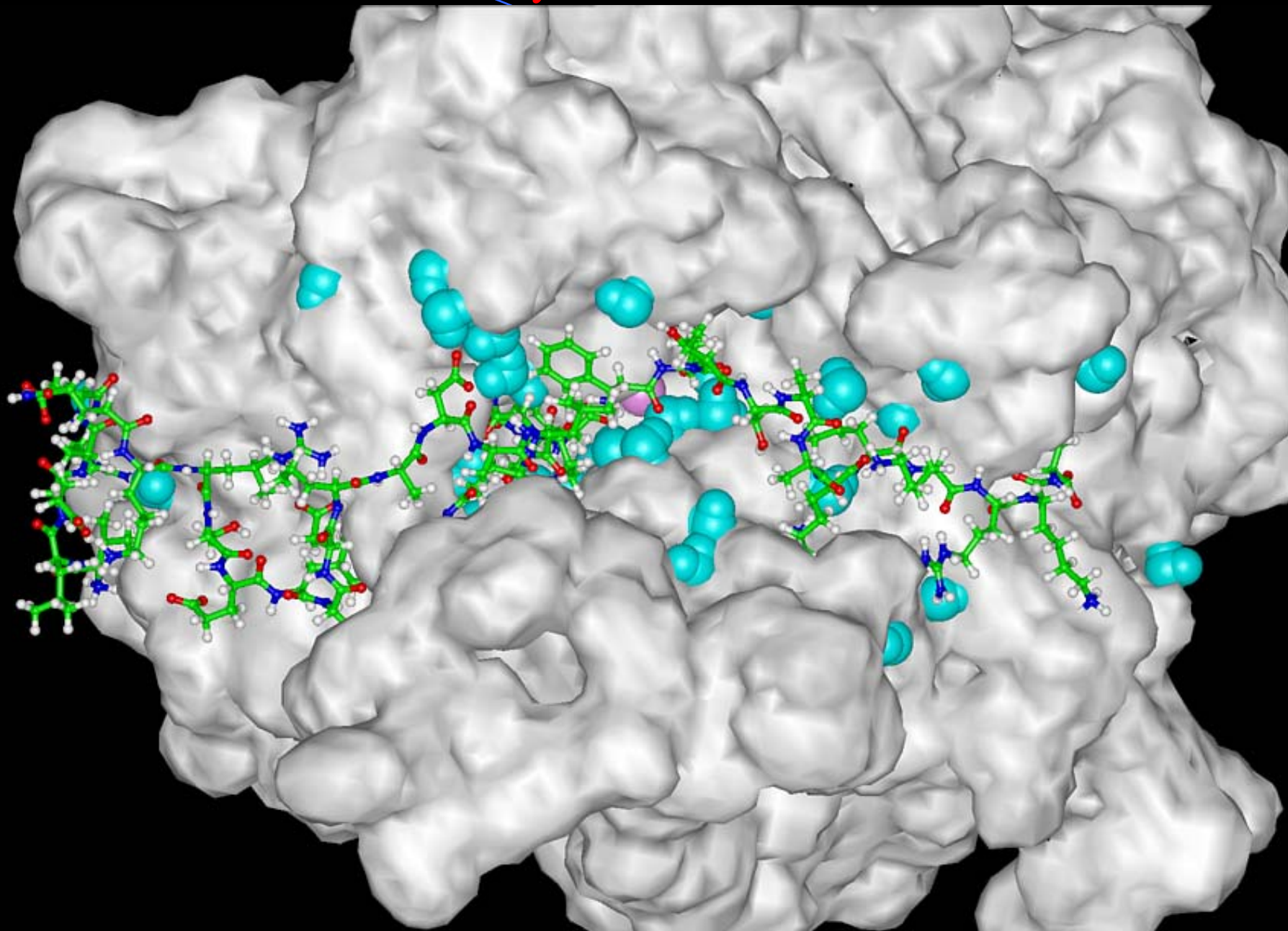


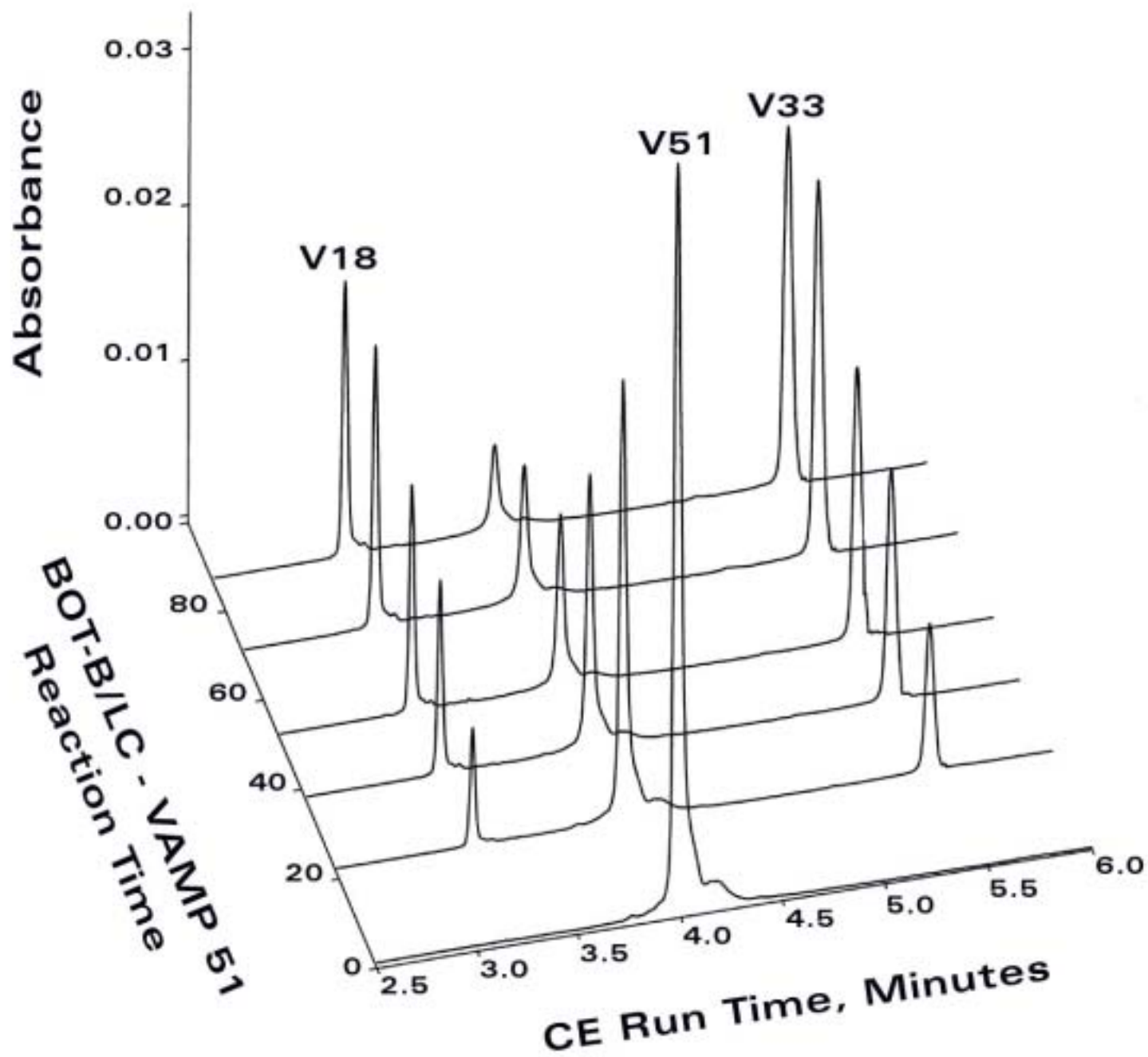


Develop *in vitro* Cleavage Assay for BoNT/B

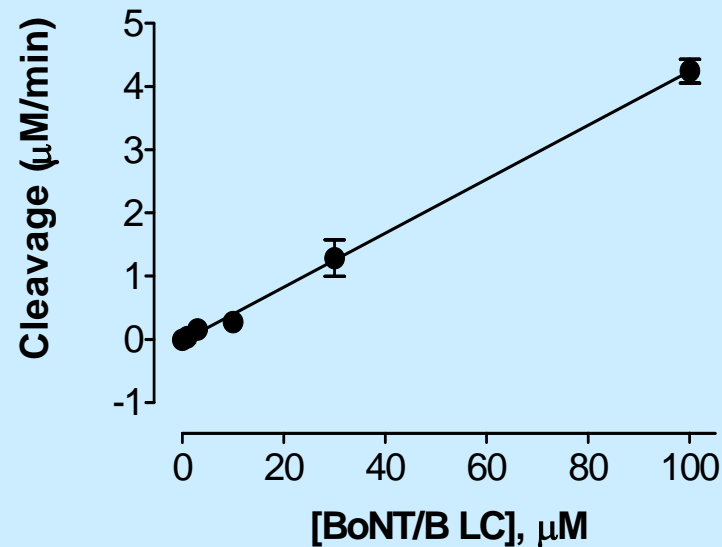
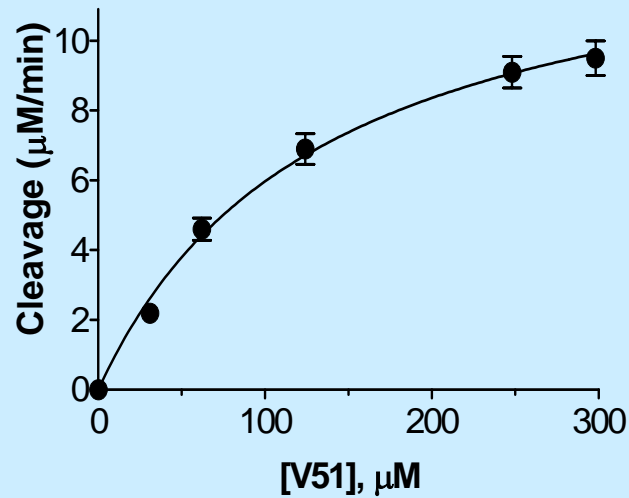
- Cleave VAMP51 (aa 44-94) with BoNT/B LC
- Monitor reaction with CE
- Test potential inhibitors by pre incubation with BoNT/B LC

VAMP-51 Co-crystallized with BoTx/B-LC

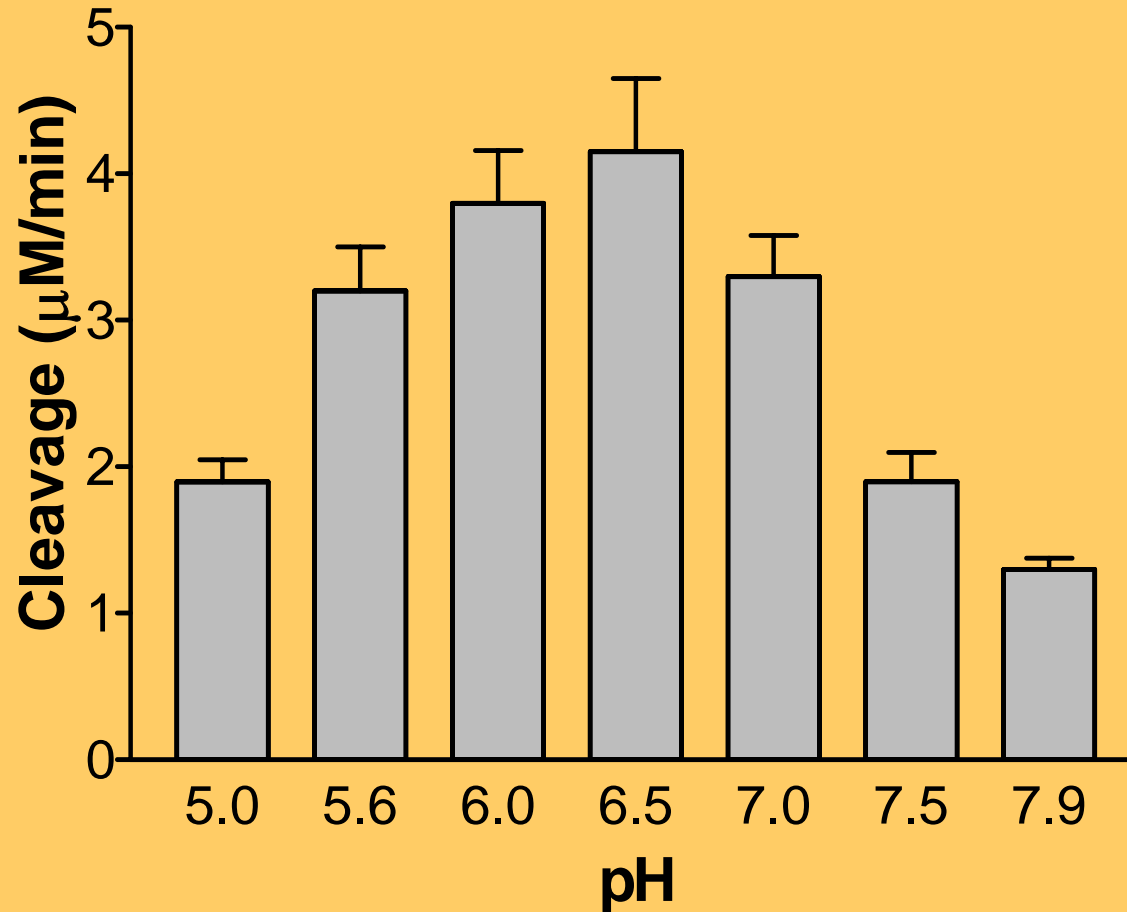




VAMP51 and BoNT/LC Concentrations

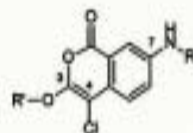


pH on VAMP51 Cleavage



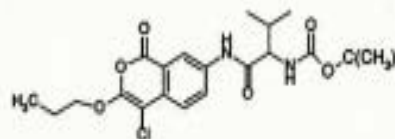
ANALOGS of ICD1578

General
Formula



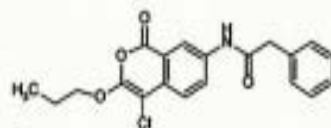
IC₅₀ (μM)

Cmpd 15



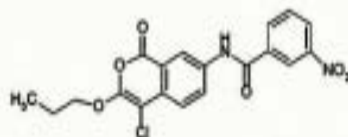
11.2

Cmpd 13



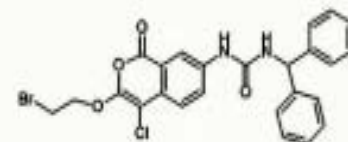
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Cmpd 16



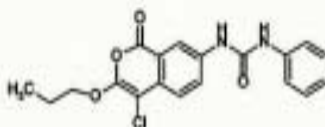
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Cmpd 44



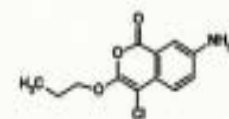
20-30

ICD 1578



27.6

Cmpd 3

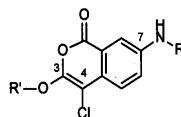


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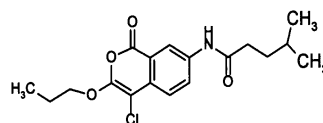
ANALOGS of ICD1578

IC50 (uM)

**General
Formula**

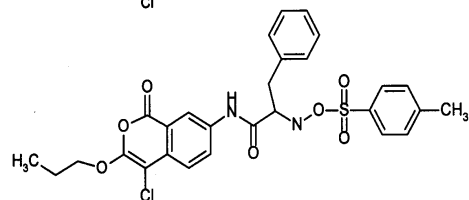


Cmpd 12



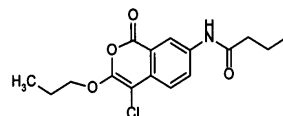
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Cmpd 21



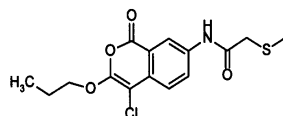
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Cmpd 11



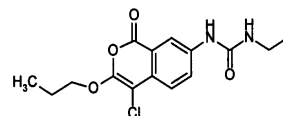
~75

Cmpd 14



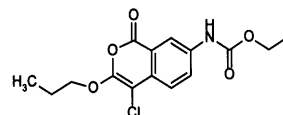
~200

Cmpd 18

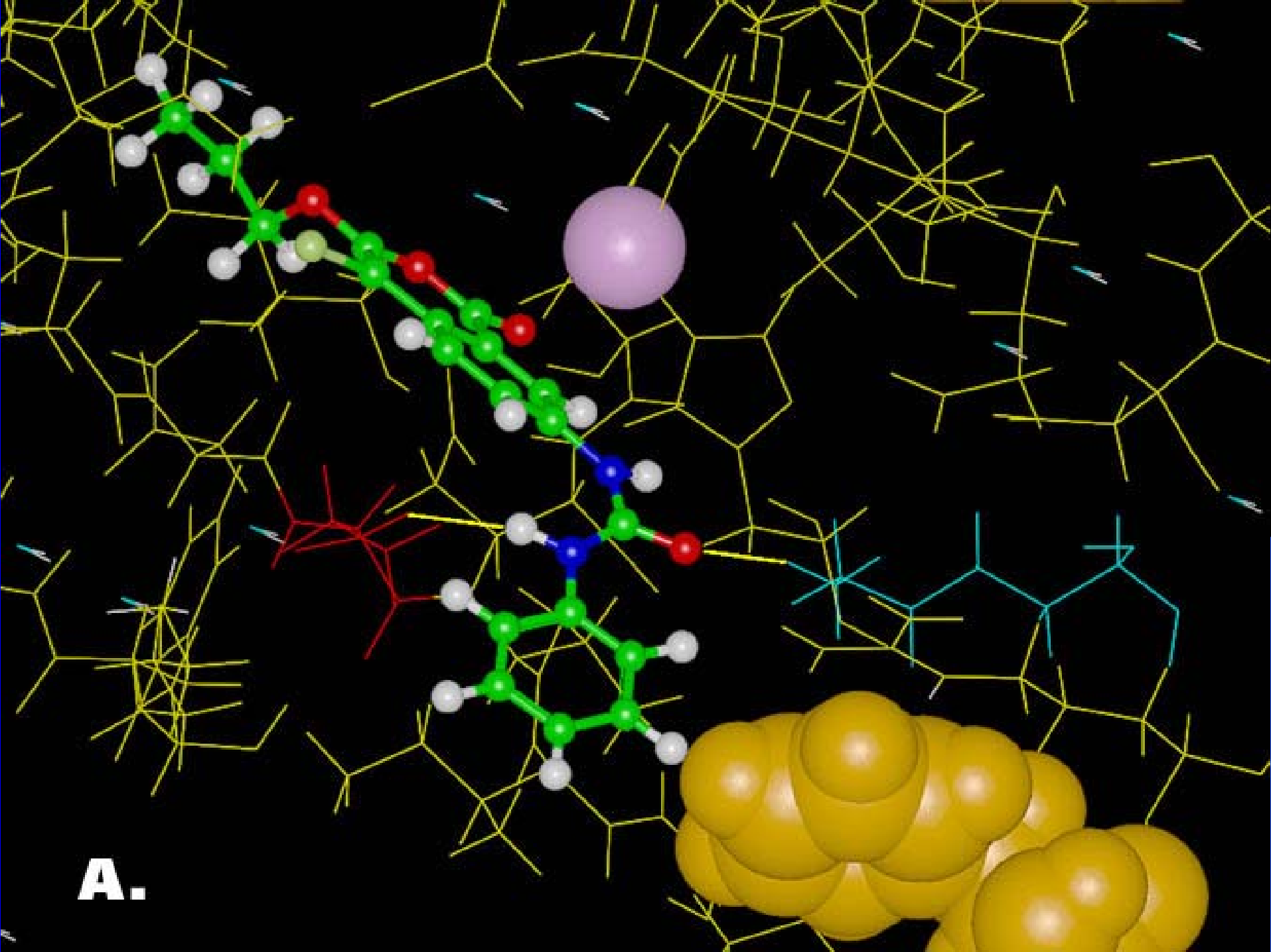


~280

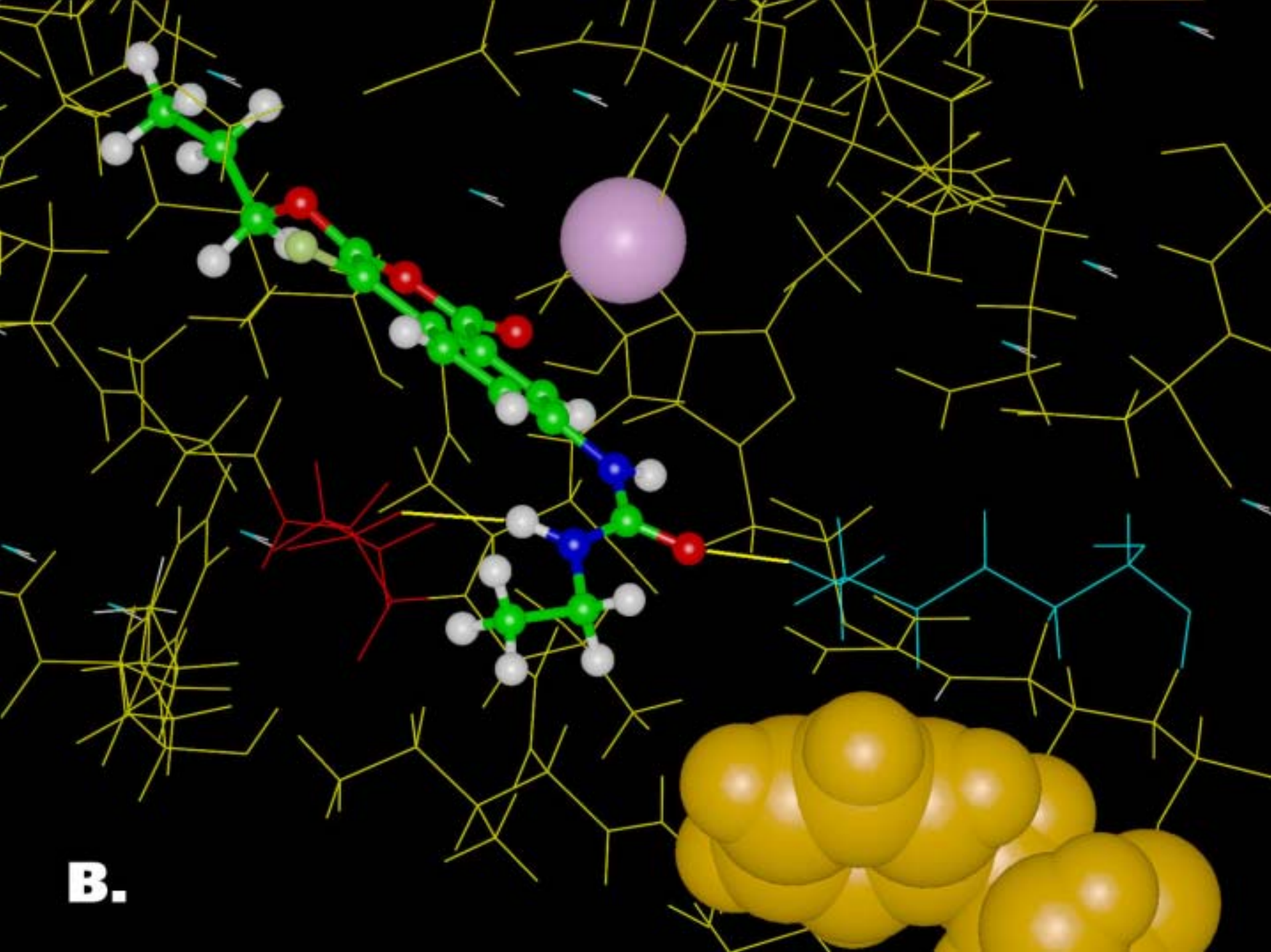
Cmpd 17



~300



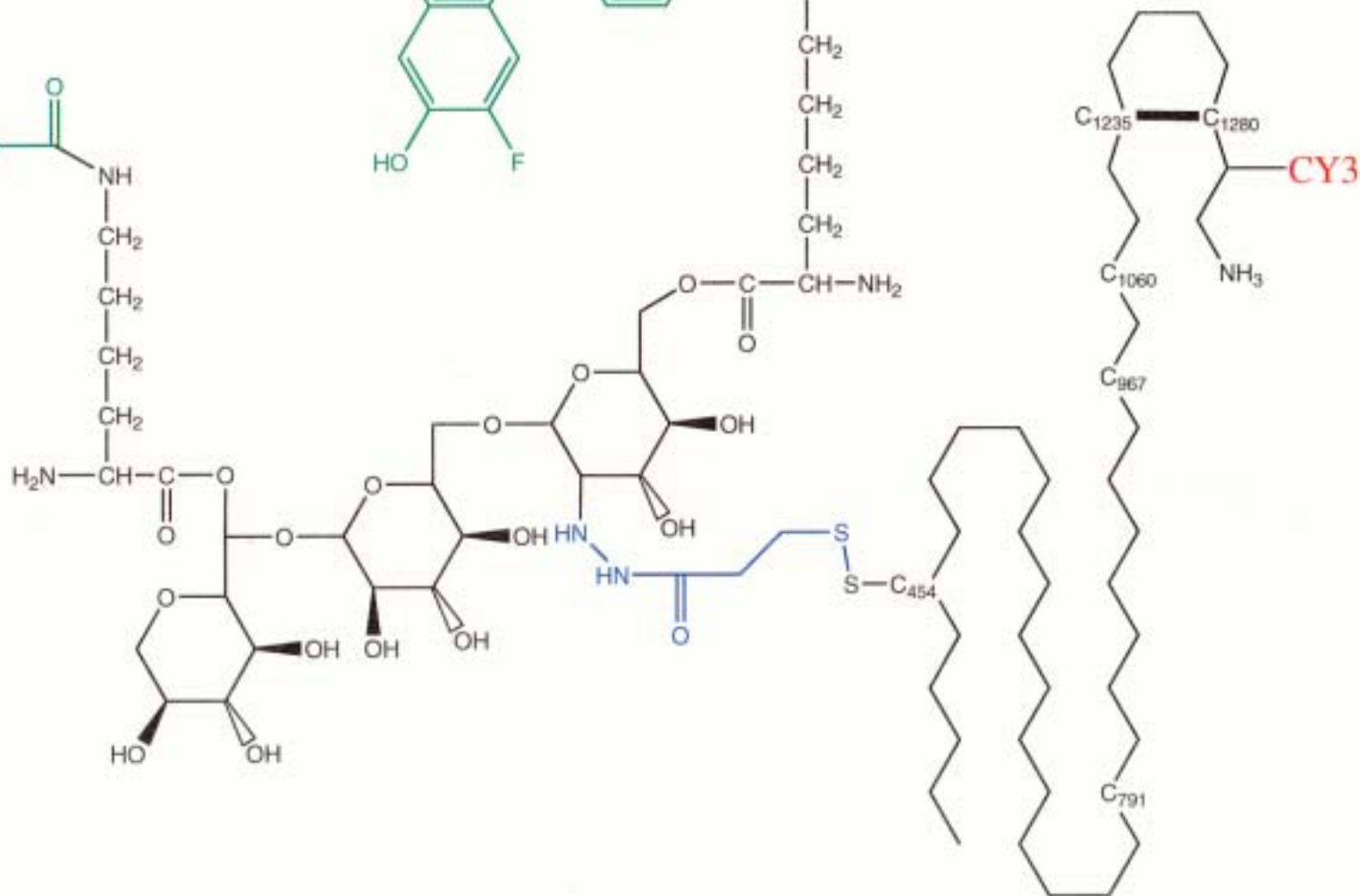
A.



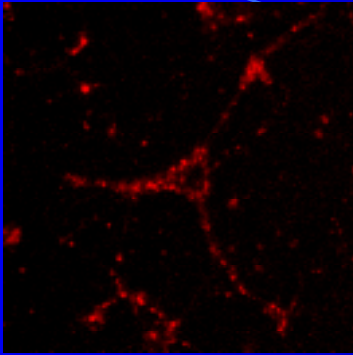
B.

Delivery Vehicle

- Target peripheral cholinergic terminals
 - BoNT HC
- Reversible binding of drugs
 - Polymer
 - High binding capacity
 - Absence of toxicity

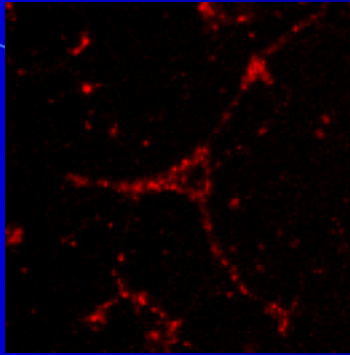
O=C1C(=C(C(=C2C(=C(C(=C1)O)F)O2)C(=C3C(=C(C(=C(C(=C3)C(=O)O)C(=O)NCCCC)C)C)C)C)FO=C1C(=C(C(=C2C(=C(C(=C1)O)F)O2)C(=C3C(=C(C(=C(C(=C3)C(=O)OCCN)C)C)C)C)C)C)F

**RED &
GREEN**



AHc-Cy3

**RED
ONLY**

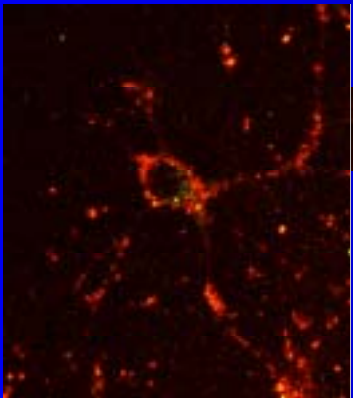


AHc-Cy3

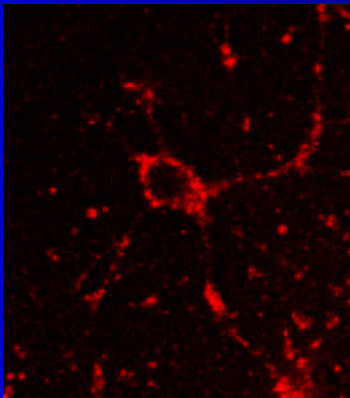
**GREEN
ONLY**



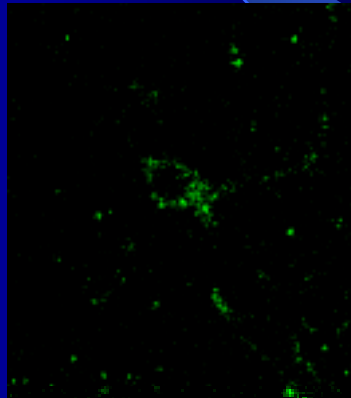
AHc-Cy3



AHc-Cy3 + 10kDa dextran-
Oregon Green 488



AHc-Cy3 + 10kDa dextran-
Oregon Green 488



AHc-Cy3 + 10kDa dextran-
Oregon Green 488



10kDa dextran-O.G.-488



10kDa dextran-O.G.-488



10kDa dextran-O.G.-488

Summary and Conclusions

- An integrated approach was developed for studying the actions of BoNT inhibitors
 - Cell free enzymatic assay
 - Cultured cells
 - Isolated tissues
- Inhibitors of BoNT binding and translocation are effective but provide only a brief therapeutic window
- A number of new protease inhibitors were identified. These will be evaluated on cultured cells and mouse diaphragm preparations
- A first generation delivery vehicle has been developed
- Improvements in inhibitors and delivery systems are expected to lead to successful pharmacological treatment of BoNT intoxication

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